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REVIEW

# Nonalcoholic Fatty Liver Disease: A Review and Update

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**Abstract** The spectrum of nonalcoholic fatty liver disease (NAFLD) ranges from asymptomatic steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. Hepatic steatosis occurs when free fatty acids, released in the setting of insulin resistance and the metabolic syndrome, are taken up by the liver. Additional biochemical insults, including oxidative stress, upregulation of inflammatory mediators, and dysregulated apoptosis, can result in inflammation (producing NASH) and fibrosis. Noninvasive methods (e.g., abdominal ultrasonography) are safe ways to support a diagnosis of hepatic steatosis, but advanced liver histopathologic findings including NASH and fibrosis cannot be identified without pursuing liver biopsy. Recent advances in serologic and imaging methods aim to determine severity of inflammation and fibrosis noninvasively. Currently, therapeutic options for NAFLD are limited to medications that reduce risk factors, but the future holds promise for therapies that might slow the progression of this increasingly prevalent disorder.

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## Introduction

NAFLD is a spectrum of disorders ranging from simple steatosis to NASH and cirrhosis. Prior to 1980, researchers documented hepatic steatosis in the livers of obese patients undergoing biopsy before or after bariatric surgery [1]. However, the term NASH was first coined in 1980 in a landmark paper by Ludwig in which he reported histological changes consisting of steatosis, inflammatory infiltrates, Mallory bodies, fibrosis, and cirrhosis in a series of 20 patients without significant history of alcohol drinking [2]. NAFLD is believed to account for up to 90% of cases of elevated liver function tests (LFTs) in patients without an identifiable cause of liver disease (e.g., viral hepatitis, alcohol, inherited liver disease, and medications) [3]. Of those who develop NASH, up to 20% of patients will develop cirrhosis over their lifetime [4]. In general, insulin resistance is implicated in the pathogenesis of NAFLD. NAFLD is immutably linked with features of the metabolic syndrome. Hence, current therapeutic approaches focus on treatment of underlying risk factors for these metabolic conditions. This review emphasizes the diagnosis and management of NAFLD.

## Epidemiology

It has been noted that NAFLD affects approximately 10–20% of the population [3]. While NAFLD is primarily a disease of adults, the condition is well described in pediatric populations [5]. Studies vary in describing prevalence of NAFLD by sex, but it is clear that both males and

females are equally affected. However, some studies reveal that NAFLD is more common in men than women in morbidly obese and Asian populations [6, 7].

While NAFLD affects both White and non-White populations, studies have also revealed that Hispanic populations have a higher prevalence of NAFLD than White and African-American (AA) populations. Furthermore, AA populations have a lower prevalence of NAFLD and cryptogenic cirrhosis compared with White populations [8, 9]. Browning et al. [8] reported that these differences may be attributable to biologic differences rather than lower rates of obesity and insulin resistance. In this study, magnetic resonance spectroscopy was used to evaluate hepatic triglyceride content (a surrogate marker of hepatic steatosis) in 2,349 subjects and showed that AA participants were less likely to develop steatosis than White and Hispanic subjects. The authors reported that inherent biological differences [e.g., lipid metabolism, as suggested by lower rates of hypertriglyceridemia and low high-density lipoprotein (HDL) cholesterol levels among AA subjects] might have contributed to this lower incidence of steatosis in AA. Recently, Stepanova et al. [10] reported genetic differences in hepatic gene expression between populations of Caucasian and African-American patients with NAFLD. This study lends further support to the assertion that inherent biological differences between racial and ethnic groups might be responsible for phenotypic variations in NAFLD.

Recently, much attention has been focused on the rising incidence of NAFLD in several Asian countries, including China, Korea, Japan, and India [11–14]. Various authors have shown that Asian patients with NAFLD display higher levels of visceral adiposity at lower body mass index (BMI) than the Caucasian population, and new BMI criteria have been created to adjust for this anthropometric difference [12, 15, 16]. Central obesity is associated with insulin resistance and may be a more important risk factor in Asian population than in Caucasian population, as evidenced by one study in a Japanese population [15]. The interesting association between location of adiposity and disease has recently been studied by Suzuki et al. [17]. They found that peripheral adiposity (measured at the arm and hip) was more predictive of fibrosis than was central adiposity (measured at the waist) among 483 patients with histologically confirmed NAFLD.

There is ample and convincing evidence describing the association between NAFLD and features of the metabolic syndrome. More advanced histologic patterns are found in liver specimens obtained from patients who meet strict criteria for the metabolic syndrome [18]. Additionally, the vast majority of patients with obesity and/or diabetes may have evidence of hepatic steatosis. Obesity has been shown to be an independent predictor of advanced fibrosis [3, 19]. Colicchio et al. [20] performed liver ultrasound in 187 obese, nondiabetic patients and found that severe steatosis was uniformly present in those with grade III obesity (defined as BMI $\geq$40 kg/m$^2$) and that in this group of patients steatosis was related to insulin resistance and serum ferritin. Central obesity (visceral adiposity) may have implications for the development of NAFLD independent of overall obesity, which is historically defined by BMI [21–23]. In addition, visceral adipocytes have been shown to be more resistant to insulin and associated with elevated levels of inflammatory mediators compared with subcutaneous adipocytes [22]. Furthermore, insulin resistance and hyperlipidemia (in particular, hypertriglyceridemia) are independently associated with NAFLD [24, 25].

Recent evidence supports an association between NAFLD and atherosclerosis. Furuta et al. [26] analyzed 38 patients with biopsy-proven NASH using several indicators of arteriosclerosis such as ankle-brachial index (ABI), pulse wave velocity (PWV) measurement, and carotid ultrasonography. Additionally, other biochemical parameters such as low-density lipoprotein (LDL) and anthropometric parameters such as calculation of visceral fat area (VFA), subcutaneous fat area (SFA), and abdominal fat area (AFA) were also measured. Liver fibrosis was associated with several markers of arteriosclerosis, while hepatic necroinflammatory grade was not associated with these markers, but rather was associated with body fat percentage and liver–spleen attenuation differences on computed tomography. Brea et al. [27] performed a case–control study in which 66 patients with ultrasonographic evidence of NAFLD were compared with a control population. Ultrasound was used to measure carotid artery intimal medial thickness (IMT). Higher levels of IMTwere suggestive of more severe atherosclerosis. Additionally, NAFLD was an independent predictor of increased IMT. The authors suggested that the proinflammatory state that accompanies the transition from steatosis to NASH might also have proatherogenic effects. Lastly, in a large study by Kim et al. [28], over 3,000 patients with ultrasonographic evidence of NAFLD underwent computed tomography to detect coronary artery calcification (CAC), a surrogate marker of coronary artery disease. In this study, NAFLD was an independent predictor of elevated CAC and there was a positive correlation between level of CAC and severity of NAFLD. Together, these three studies highlight the association between NAFLD and cardiovascular disease, suggesting that similar inflammatory mediators invoke damage to hepatic parenchyma and peripheral vasculature. Physicians should heed this association and aim for early detection and reduction of risk factors that contribute to NAFLD and atherosclerosis.

## Natural History and Prognosis

Steatosis and fibrosis have been described as histological changes within the liver and commonly exist in patients who remain asymptomatic. Fassio et al. [29], in a prospective study, demonstrated that 30% of patients with NASH show histological progression of fibrosis within 5 years. Furthermore, of patients with NASH, up to 15–20% may develop cirrhosis and, of these patients who develop cirrhosis, 30–40% may suffer liver-related mortality [4]. One of the largest studies to date on the natural history of NAFLD included 435 patients in Olmsted County, Minnesota, over a mean follow-up of 7.6 years [30]. The retrospective analysis of this study revealed that 13 patients (3%) initially diagnosed with NAFLD went on to develop cirrhosis. The leading causes of death in this study were heart disease and malignancy, followed by liver-related mortality [seven patients (1.7%)]. None of the patients diagnosed with simple steatosis developed cirrhosis or liver-related complications over 7 years of follow-up. Even though a benign course in patients with steatosis has been described elsewhere, many studies on the natural history of NAFLD are limited by small sample size and limited duration of follow-up [31, 32]. Chalasani [33] suggests that these limitations make it difficult to conclude that steatosis is a benign phenomenon. In fact, up to 5% of patients may progress from steatosis to NASH and even cirrhosis [34]. In further support of the notion that steatosis by itself has profound clinical ramifications is the finding, reported by Tarantino et al. [35], of similar levels of transforming growth factor-beta 1 in serum of patients with steatosis as well as NASH. This data suggests that these two seemingly disparate conditions share common pathophysiology and that "simple steatosis" might not be benign. Given the fact that patients with NASH can enter a final cirrhotic pathway similar to that in patients with alcoholic cirrhosis or in patients suffering from chronic hepatitis B or C, it is not surprising that NASH appears to portend an increased risk of hepatocellular carcinoma (HCC) as well [36]. Recently, Yatsuji et al. [37] followed 24 patients who underwent curative therapy for HCC in the setting of NASH and found that 88% had recurrence of HCC after treatment, predicted by age at diagnosis of HCC, treatment, male gender, and stage of fibrosis. This recurrence rate of HCC in patients with NASH is higher than in patients without NAFLD (70%), which might influence clinicians to pursue earlier and more aggressive therapy for HCC in the setting of NAFLD [38]. This suggests that the underlying process resulting in HCC in NASH may be related to a multisystemic disorder, including the metabolic syndrome.

## Pathogenesis

Insulin resistance, oxidative stress, and an inflammatory cascade are believed to play integral roles in the pathogenesis and progression of NAFLD. A "multi-hit" (formerly "double-hit") hypothesis has been used to describe the pathogenesis of NAFLD (Fig. 1) [39, 40]. Insulin resistance results in increased elaboration of free fatty acids (FFA) that are absorbed by the liver, resulting in steatosis—the first "hit." Superimposed upon this background of hepatic steatosis is a series of complex interactions (i.e., multiple "second hits") between hepatocytes, stellate cells, adipose cells, Kupffer cells, inflammatory mediators, and reactive oxygen species that result in inflammation (NASH) or cirrhosis.

Insulin resistance initiates the first "hit." In insulin-resistant states, adipose and muscle cells preferentially oxidize lipids, resulting in the release of FFA. FFA can then be taken up by the liver, resulting in steatosis. It is still unclear why some patients progress past a steatosis stage and develop inflammation and fibrosis. However, animal studies provide insight into this mechanistic conundrum. FFA, once released from muscle and adipose cells, can be incorporated into triglycerides in the liver or undergo oxidation in mitochondria, peroxisomes or microsomes.

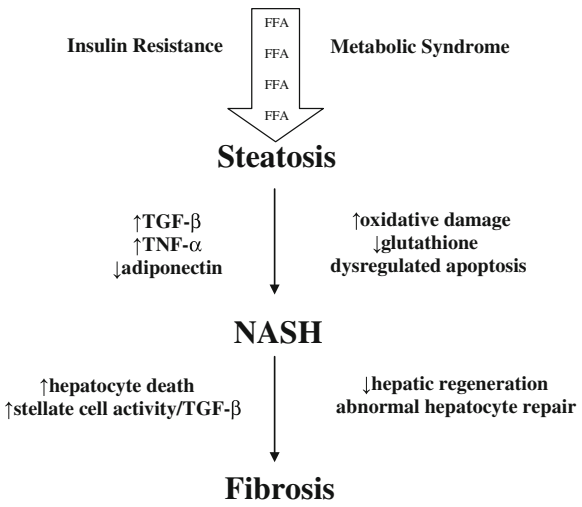


**Fig. 1** "Multi—hit" hypothesis: pathogenesis of NAFLD. In this model, dysregulated free fatty acid (FFA) metabolism (the "first hit") results in lipid accumulation in the liver, producing steatosis. Inflammatory mediators, reactive oxygen species, and abnormal apoptotic mechanisms serve as "second hits" that result in superimposed inflammation (NASH). The interplay between inflammatory mediators and the activation of stellate cells can lead to fibrosis that, left unchecked, will overcome the regenerative capacity of the liver, producing cirrhosis. (Adapted from: Edmison et al. Pathogenesis of non-alcoholic steatohepatitis: human data. *Clin Liver Dis* 2007 Feb; 11(1): 75–104, ix.)

Oxidized by-products are harmful adducts that can cause liver injury, resulting in subsequent fibrosis [4]. Lipid peroxidation and oxidative stress result in increased production of hydroxynonenal (HNE) and malondialdehyde (MDA) that upregulate liver fibrosis via activation of stellate cells and result in increased production of transforming growth factor-beta (TGF-$\beta$) [41]. As will be detailed below, overexpression of uncoupling proteins has been associated with a reduction in generation of reactive oxygen species and Kupffer cell activation, which might attenuate injury in NAFLD. In addition to insulin resistance, several authors have shown that leptin contributes to an insulin-resistant state and might even stimulate fibrogenesis in animal models of NAFLD [42, 43].

Inflammatory mediators have been implicated in the progression of NAFLD and are the focus for new therapeutics. Proinflammatory transcription factors such as nuclear factor kappa beta (NF-$\kappa$B) are often elevated in patients with NASH [44]. Adiponectin and tumor necrosis factor-alpha (TNF-$\alpha$) are two proinflammatory proteins implicated in the pathogenesis of NAFLD. Adiponectin is a hormone released from adipose cells that decreases fatty acid oxidation and inhibits hepatic gluconeogenesis [45]. Both human and mouse models have demonstrated that lower adiponectin levels are associated with increased severity of hepatic inflammation and that repletion of adiponectin in mice resulted in significant improvements in steatosis and inflammation [46, 47]. TNF-$\alpha$ is an inflammatory mediator largely produced by macrophages, but also elaborated by other cells including adipocytes and hepatocytes [39, 48]. Elevated levels of TNF-$\alpha$ have been detected in obese patients with insulin resistance and also in patients with NASH [49–51]. TNF-$\alpha$-mediated hepatic injury results from inhibition of mitochondrial electron transport and release of reactive oxygen species that stimulate lipid peroxidation [48].

Recently, scientists have focused on the role of Kupffer cells in the pathogenesis of NAFLD. Kupffer cells are the resident macrophages of the liver and function in both innate and adaptive immunity as active phagocytosing agents and antigen-presenting cells (via toll-like receptors, among others) to T-cells. While inactivation of Kupffer cells is associated with NAFLD and impaired hepatic regenerative capacity, elimination of resident Kupffer cells has been associated with improvement of NASH, suggesting that overactivation of a Kupffer-cell-mediated immune response might underlie liver injury in NAFLD. It is thought that Kupffer cell physiology becomes altered in the setting of increased hepatic lipid content possible due to overcrowding of liver sinusoids resulting in prolonged exposure of Kupffer cells to antigens, reduced Kupffer cell outflow, and a resulting sustained inflammatory response. Uncoupling proteins, molecules that dissipate the proton gradient in the inner mitochondrial membrane and thereby reduce energy needed for oxidative phosphorylation. Insufficient uncoupling protein production in Kupffer cells, possible due to lipopolysaccharide-induced activity, might contribute to the pathogenesis of NAFLD [52].

While NAFLD is histologically defined by the presence of hepatic fat, evidence suggests that NAFLD is also characterized by aggressive catabolic events and suboptimal hepatic defenses. The proapoptotic gene *Bax* is upregulated in patients with NASH and alcoholic liver disease [53]. Additionally, caspase levels, by-products of cellular apoptosis, are also increased in these groups of patients [53]. Glutathione levels, which serve as a marker of an organism's antioxidant capacity, are decreased in patients with NASH [54].

Two other seemingly unrelated processes have been studied in relation to NAFLD. Iron overload has also been implicated in the pathogenesis of NAFLD, and hyperferritinemia, which may contribute to insulin resistance, is found in one-third of patients [27, 55]. Interestingly, one study reported that hyperferritinemia, but not hepatic iron overload, was associated with more severe fibrosis [56]. Recently, the role of retinol binding protein 4 (RBP4) has been a focus of investigation for its potential role in the pathogenesis of NAFLD. RBP4 is produced by adipocytes and is implicated in the development of insulin resistance [57]. Studies have revealed that elevated RBP4 is an independent predictor of the development of NAFLD [58, 59]. Recently, Petta et al. [60] found that patients with hepatitis C and steatosis and patients with NAFLD had higher levels of RBP4 compared with normal controls. In the subset of patients with NAFLD, RBP4 levels were closely associated with insulin resistance and obesity. This suggests a role for RBP4 as an adipocytokine contributing to NAFLD and a potentially useful marker of steatosis [61].

## Diagnosis

A diagnosis of NAFLD can be achieved after excluding other causes of abnormal liver function tests (LFTs) and after performing appropriate imaging. In the clinical setting, there is no consensus about whether or not liver biopsy is required to confirm a diagnosis of NAFLD.

### History and Physical Examination

The majority of patients diagnosed with NAFLD are asymptomatic. These patients are frequently identified after undergoing imaging studies for evaluation of LFTs. Patients might experience vague right upper quadrant abdominal pain, nausea, and other nonspecific symptoms referred to the gastrointestinal tract. Once NASH has

progressed to frank cirrhosis, findings seen in cirrhosis from other chronic liver diseases (e.g., spider angiomas, palmar erythema, caput medusae, ascites, and jaundice) might become apparent.

## Serology

There is no single biochemical marker that can confirm a diagnosis of NAFLD or distinguish between steatosis, NASH, and cirrhosis. Several authors have proposed measurement of novel markers to aid in the diagnosis of NAFLD, but these investigations have been limited by lack of reproducibility or inability to differentiate steatosis reliably from more advanced inflammation and fibrosis.

LFT abnormalities are common in patients with NAFLD, with elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) usually no greater than four times the upper limit of normal and a variable AST/ALT ratio, although ALT usually predominates [62, 63]. A ratio of AST/ALT >2 suggests alcoholic liver disease in addition to NAFLD. In addition, some patients can also present with isolated elevations of alkaline phosphatase [64].

Several authors have described noninvasive models that aim to differentiate simple steatosis from NASH. Pelekar et al. [65] utilized 8-epi-$PGF_{2\alpha}$, TGF-$\beta$, hyaluronic acid (HA), and adiponectin in a model that predicted NASH with favorable sensitivity (73.7%), specificity (65.7%), positive predictive value (68.2%), and negative predictive value (68.2%). Poynard et al. [66] proposed the SteatoTest, a battery of biochemical markers including ALT, $\alpha_2$-macroglobulin, apolipoprotein A-I, haptoglobin, total bilirubin, $\gamma$-glutamyl transpeptidase (GGT), cholesterol, triglycerides, glucose, age, gender, and BMI, that predicted >30% steatosis with sensitivity of 90%, specificity of 90%, negative predictive value of 93%, and positive predictive value of 63%.

Several studies have been conducted regarding serological models that might support a diagnosis of advanced fibrosis in NASH or cirrhosis. It is worthwhile to study predictors of fibrosis because patients who have elevated markers of fibrosis might be at risk for developing cirrhosis and the complications thereof. The FibroTest includes $\alpha_2$-macroglobulin (A2 M), apolipoprotein A-I, haptoglobin, total bilirubin, $\gamma$-glutamyl transpeptidase (GGT), and ALT [67]. This test displays strong positive (73%) and negative (90%) predictive value for severe fibrosis, but does not differentiate among stages of fibrosis. Similarly, the NAFLD fibrosis score, an index consisting of age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio, showed strong positive (82%) and negative (93%) predictive values for advanced fibrosis in NAFLD [68]. A comparison of Hepascore (bilirubin, $\gamma$-glutamyltransferase, hyaluronic acid, $\alpha_2$-macroglobulin, age, and sex), Fibrotest, and AST-to-platelet ratio index (APRI, initially used as a marker of fibrosis in patients with hepatitis C) showed that each index could accurately predict significant (F2–F4) and advanced fibrosis (F3–F4), but that the APRI was a less accurate predictor of cirrhosis (F4) [69]. Recently, the BARD score has been validated as a predictive tool in assessing fibrosis in patients with NAFLD. Harrison et al. [70] studied 827 patients with NAFLD and found three variables, BMI $\geq$28 kg/m$^2$, AST/ALT ratio $\geq$0.8, and type II diabetes, that together predicted advanced fibrosis with an odds ratio (OR) of 17 and a negative predictive value of 96%.

Many groups have sought to detect correlations between markers of oxidative stress and histologic disease. Thiobarbituric acid (TBAR) derivatives and oxidized LDL are by-products of oxidative stress. While Horoz et al. showed that patients with steatosis alone and NASH have higher levels of these markers, on the other hand, Bonnefont [71, 72] and his group showed no correlation between fibrosis and markers of oxidation (e.g., TBAR and superoxide dismutase) or markers of antioxidant capacity (e.g., glutathione, vitamin E, and selenium).

Inflammatory mediators involved in the "multi-hit" hypothesis have also been investigated as potential diagnostic tools. Diminished levels of adiponectin have been observed in patients with NAFLD [4, 73]. In a mouse model, supplementation of adiponectin resulted in improvement in steatosis and reduction in TNF-$\alpha$ levels [47]. Several authors have studied the relationship between leptin, a hormone secreted from adipose tissue, and liver histology. Animal models have shown that leptin has profibrotic effects [42]. Even though human studies reported that elevated leptin levels are found in patients with steatosis and NASH, absolute leptin levels have not been shown to correlate with degree of steatosis or fibrosis [74–76]. Not only are TNF-$\alpha$ levels elevated in patients with NASH, but absolute levels are correlated with severity of inflammation and fibrosis [49, 77]. Other circulating markers of inflammation, in particular interleukin-6 (IL-6), CC-chemokine ligand 2 (CCL-2), and hyaluronic acid (HA), are elevated in patients with NASH [78–80]. HA levels are also associated with patients that have developed progressive fibrosis [81]. Lastly, N-glycans, glycoproteins that modify proteins during passage through the endoplasmic reticulum, have been used to predict severity of NAFLD. Callewaert et al. [82] sequenced the N-glycan profile of 248 patients. Using two such N-glycan moieties (the combination referred to as the GlycoCirrhoTest), in combination with the FibroTest (described above), this group was able to differentiate cirrhotic livers from noncirrhotic livers with 100% specificity and 75% sensitivity. Enzymes responsible for N-glycan modification are found in regenerating hepatic parenchymal cells, whereas healthy parenchyma does not express this

enzyme. Thus, the authors concluded that the modified N-glycans found in this study are associated with regenerating and/or cirrhotic livers. More recently, Schmilovitz-Weiss [83] showed that N-glycan levels were increased in patients with NAFLD and were associated with increased levels of fibrosis.

Dysregulated apoptosis is implicated in the pathogenesis of NAFLD (as mentioned above via overexpression of the *Bax* gene), and researchers have examined whether measurement of proteins released during this process can be used to differentiate steatosis from more severe histological patterns of NAFLD [53]. Feldstein et al. [84] showed that mice with evidence of fatty liver had elevated levels of fas ligand, a transmembrane protein that interacts with fas ligand receptor to induce apoptosis. Wiekowska and Feldstein [85] demonstrated that cytokeratin-18 (CK-18) fragments, remnants of the cellular cytoskeleton that become available after caspase cleavage during apoptosis, were increased in patients with NASH compared with patients with steatosis only. Tarantino et al. [86] showed that tissue polypeptide specific antigen, a protein released in proportion to cytokeratin-18 during apoptosis, is a more accurate marker of fibrosis than alanine aminotransferase levels. Other studies have shown that CK-18 fragments can reliably distinguish between early and severe fibrosis [87, 88]. More recently, Tabesh et al. [89] reported that patients with NASH treated with metformin and pioglitazone had lower levels of CK-18 fragments after therapy, suggesting that these fragments might be followed when assessing response to therapy. Additional studies are needed to confirm the predictive value of apoptotic markers for the diagnosis of NAFLD.

## Imaging

Ultrasound is the least expensive modality for detecting changes associated with NAFLD. The radiologic correlate of steatosis in ultrasound is increased echotexture, or a "bright" liver. However, ultrasound is less accurate for detection of steatosis when fat content in the liver falls below 30% [90]. Saadeh et al. [90] reported the sensitivity of ultrasound and computed tomography (CT) scan to be 100% and 93%, with positive and negative predictive values of 62% and 76%, respectively (Table 1). In a population of 187 obese patients undergoing bariatric surgery, ultrasound diagnosed steatosis with 49.1% sensitivity and 75% specificity [91]. Another study by Palmentieri et al. [92] of 235 patients undergoing ultrasound with liver biopsy showed that a "bright liver" pattern on B-mode ultrasonography showed sensitivity, specificity, positive predictive value, and negative predictive values of 91%, 93%, 89%, and 94%, respectively, for predicting steatosis of >30%. However, bright liver contrast was not associated with fibrosis in this study.

Some authors suggest that hepato-renal contrast more accurately predicts steatosis and fibrosis [93, 94]. Nonsteatotic hepatic parenchyma exhibits an echotexture similar to that of renal parenchyma, but becomes "brighter" when infiltrated with fat, which serves as the basis for hepato-renal contrast [95]. Webb et al. [96] studied 93 patients with chronic liver disease who underwent liver biopsy and found that hepato-renal index could quantify severity of steatosis (to a lower limit of 5%). Osawa and Mori [95] used CT scan as the gold standard and found that hepato-renal difference predicted steatosis with 91.3% sensitivity, 83.8% specificity, and 86.7% accuracy. In a study of 375 patients with liver disease (45 receiving biopsies), steatosis measured by hepato-renal contrast was significantly more sensitive (100%) and specific (100%) for detecting >5% steatosis compared with traditional ultrasonography (70% and 84%, respectively). However, inflammation and fibrosis were not measured in any of these studies. Additionally, these studies were performed in heterogeneous

**Table 1** Noninvasive diagnosis of NAFLD

| Modality | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Comments |
|---|---|---|---|---|---|
| Ultrasound [85–91] | 91–100 | 93–100 | 62–89 | 94 | Best for detecting >30% steatosis. Unable to detect inflammation or fibrosis |
| Ultrasound (Levovist) [92] | 100 | 95–100 | N/A | N/A | Microbubble accumulation not correlated with fibrosis or steatosis |
| Ultrasound (liver elasticity) [102, 103] | 91 | 84 | 47 | 97 | Able to detect differences between severe (F3-F4) and mild (F1-F2) fibrosis |
| CT [94–96] | 93 | N/A | 76 | N/A | Unenhanced study preferred |
| MRI [98] | N/A | N/A | N/A | N/A | Can detect steatosis of 3% |
| MR (spectroscopy) [99, 100] | N/A | N/A | N/A | N/A | In one trial validating method, reported population-wide prevalence of steatosis at 34% |
| MR (elastography) [104–106] | 85 | 86 | 73 | 94 | Changes in viscosity and elasticity preceded biopsy findings of fibrosis |

*N/A* not available, *NPV* negative predictive value, *PPV* positive predictive value, *MRI* magnetic resonance imaging, *MR* magnetic resonance

populations of patients with hepatitis, alcoholic liver disease, and other causes of elevated LFTs, which may make the results less applicable to the NAFLD population.

One criticism of ultrasonography is that it has failed to prove efficacious for detection of inflammation and fibrosis. In a recent study, however, Iijima et al. [97] used an ultrasound contrast agent (Levovist; Sherling, Berlin) to detect NASH. Levovist contains galactose and palmitic acid and is taken up by hepatocytes. These moieties participate in sugar and fat metabolism [98]. Patients with NASH had significantly reduced uptake of Levovist [97]. This finding is not surprising given that patients with NAFLD have higher circulating levels of free fatty acids that do not participate in metabolism but rather mediate cellular injury. Larger studies are required to evaluate contrast ultrasonography for use in the diagnosis of NASH and advanced fibrosis.

CT scan technology has also been used to evaluate liver architecture. In a study by Piekarski et al. [99] in which they measured noncontrast CT numbers of normal subjects, the average CT number of the liver was 24.9 Hounsfield units (HU) (range 16.7–37.2 HU) and the average CT number of the spleen was 21.1 HU (range 14.9–34.3 HU). In this study, fatty livers were associated with lower CT numbers. Park et al. [100] utilized nonenhanced CT to detect steatosis in 154 subjects who also underwent liver biopsy and used liver-to-spleen attenuation ratio and liver-to-spleen attenuation difference to detect steatosis >30%. They reported specificity of 100%, but sensitivity varied from 73% to 82% for the indices used above. In another study including 703 live liver donor candidates, Lee et al. [101] studied liver-to-spleen attenuation and visual grading on nonenhanced CT scans and compared these values with findings at liver biopsy. Both measures proved accurate for the diagnosis of steatosis >30%. It appears that noncontrast CT scan appears to be more useful for detecting steatosis than contrast-enhanced scans [102].

Magnetic resonance imaging (MRI) has been shown to detect steatosis reliably. Fatty changes are assessed by differential chemical shifts between fat and water. Fishbein et al. [103] reported good correlation between MRI, ultrasound, and histology in patients with NAFLD. MRI, however, has been shown to more accurately detect lower levels of steatosis than those detected by ultrasonography and CT scans, as illustrated in a study by Fishbein et al. [103], who showed that MRI was able to detect steatosis of 3%. A variant of MRI, proton magnetic resonance spectroscopy (MRS), has been shown to measure steatosis accurately [104]. Findings from proton MRS correlate with ultrasonographic findings of steatosis. Szczepaniak et al. [105] used proton MRS to measure hepatic triglyceride levels (HTGC) in 375 subjects. In the validation portion of this study, 34.3% of the 2,287 participants studied had HTGC >5%, a level deemed diagnostic of hepatic steatosis. Interestingly, the prevalence of steatosis in their study population was 37.6%, which was similar to the estimated population prevalence of steatosis as reported by Browning et al. [8]. While MRI and MRS can detect subtle changes in fat more accurately than ultrasound or CT, these technologies are more expensive and less accessible than their counterparts.

Liver elasticity is another noninvasive measure of liver fibrosis. When ultrasound is used, an ultrasound probe emits a vibration that creates a shear wave within the liver. This shear wave corresponds to liver stiffness [106]. Takeda [107] compared liver stiffness as measured by FibroScan (ultrasound) in 27 patients with biopsy-proven NAFLD and found that the stiffness score was correlated with Brunt fibrosis score. In addition, liver stiffness was significantly higher in patients with stage 3 or 4 fibrosis than lower stages. In a larger study, Fukuzawa et al. [108] measured liver elasticity in a series of 135 patients with biopsy-proven NASH and showed that liver elasticity accurately predicted fibrosis. Furthermore, elasticity measurement was able to distinguish subjects in each of the Brunt classification stages of fibrosis (F0-1, F2, F3, and F4). Liver stiffness has also been measured using MRI technology [109]. In magnetic resonance elastography (MRE), a mechanical wave is created and MRI technology is used to measure liver displacement, which can be converted to a measure of elasticity [110]. Yin et al. [111] performed MRE in 85 patients and found that this modality was able to differentiate stage 2–4 fibrosis from stage 0–1 fibrosis with sensitivity of 86% and specificity of 85%.

Another novel ultrasound-guided method of assessing abnormalities within hepatic parenchyma NAFLD is the Doppler perfusion index (DPI), a ratio of hepatic arterial blood flow to total liver blood flow. Hepatic hemodynamics are altered when space-occupying lesions are present within the parenchyma [112]. For example, elevated DPI has been shown to be present in patients with metastatic colorectal cancer [112]. In small trials, altered DPI has been found in patients with NAFLD [113–115]. However, the full results were not disclosed. As this technology has only been applied for the diagnosis of NAFLD in small trials, larger studies will need to be performed before DPI becomes commonplace in clinical practice.

## Liver Biopsy

NAFLD is characterized by macrovesicular steatosis, lobular inflammation, Mallory bodies, ballooning degeneration, and perisinusoidal/perivenular fibrosis. Several systems have been devised to systematically characterize the histology of NAFLD. In 1999, Brunt developed a classification system utilizing ten histological variables to

create a necroinflammatory grade and fibrosis score [116]. More recently, the NASH Clinical Research Network (CRN) devised a new system of staging of NAFLD [117]. The NASH CRN system was necessary because the earlier system created by Brunt was intended for patients with NASH and was not able to fully characterize the histological spectrum of NAFLD (from steatosis to NASH and fibrosis). Measures in the NASH CRN criteria include the following: steatosis, lobular inflammation, hepatocyte ballooning, Mallory bodies, and fibrosis. Together, these variables are used to calculate a NAFLD Activity Score (NAS) that is used to distinguish steatosis from NASH in clinical trials. However, NAS score does not include fibrosis, and fibrosis is reported separately. A NAS score greater than or equal to 5 is likely to represent NASH, a score of 0–2 is unlikely to represent NASH, and a score of 3 or 4 is indeterminate (some patients show evidence of NASH). Fibrosis is scored separately from 0 to 4: a score of 0 is applied to biopsy specimens without evidence of fibrosis and a score of 4 represents cirrhosis.

Is a liver biopsy necessary to confirm the diagnosis of NAFLD? The diagnosis of NAFLD can usually be confirmed by a combination of history, serologies, and abdominal imaging. However, liver tissue is needed to determine the severity of NAFLD. One author argues that a benefit of biopsy would be the discovery of advanced fibrosis or cirrhosis that would result in earlier endoscopic screening for varices and monitoring for complications of cirrhosis [118]. In addition, patients with advanced fibrosis and cirrhosis might be considered for screening for HCC [119]. Furthermore, older age and diabetes are independent predictors of fibrosis, and biopsy in such at-risk populations might detect advanced disease earlier. Hence, it is conceivable that the presence of advanced fibrosis or cirrhosis will encourage physicians and patients to aggressively manage risk factors such as hypertension, diabetes, dyslipidemia, and obesity. On the other hand, there are limited therapeutic options for patients with steatosis and NASH. Further complicating matters, sampling error confounds the results of liver biopsy. In a series of 51 patients who underwent two separate liver biopsies, 20% of cases would have been misdiagnosed as steatosis only instead of NASH had the second biopsy not been performed [120]. The authors contend that this finding might have diagnostic and treatment ramifications in the future as therapeutic options for NAFLD are discovered.

## Treatment

Unlike other chronic diseases of the liver (e.g., chronic hepatitis C), there are no formal algorithms that simplify management of NAFLD. Currently, management of NAFLD consists of modification of underlying risk factors, detection of patients that have progressed to cirrhosis, management of cirrhosis-related morbidity, and transplantation in patients with end-stage liver disease. While some agents show modest improvements in LFTs and even histologic parameters, studies performed to date are limited by small sample sizes and short follow-up periods. Until larger, randomized-controlled trials are performed, agents mentioned below should generally be used to modify risk factor profiles rather than as primary therapy for NAFLD.

### Weight Loss

Lifestyle and surgical management of obesity have beneficial effects on several parameters of the metabolic syndrome and have generally been associated with improvement in steatosis.

#### *Diet and Exercise*

Behavioral therapy, diet, and exercise have been shown to result in significant improvement in LFTs in obese patients [121–123]. In a series of 25 obese patients randomized to a restrictive diet and exercise program, patients in the intervention group had lower ALT values and improvement in steatosis after 3 months [124]. Calorie restriction also produced improvements in histology in a study by Huang et al. in which participants received nutritional counseling and were limited to a 1,400 kcal/day diet. Nine of 15 participants who underwent postintervention biopsy showed improvements in hepatic steatosis [125]. Sato et al. [126] measured intrahepatic lipid content using MR spectroscopy and found that nondiabetic, obese subjects showed significantly lower levels of intrahepatic lipids after 3 months of a caloric-restricted diet. Bonekamp et al. [127] reported improvement in MR-spectroscopy-defined levels of steatosis (2.5%) after 6 months of controlled exercise in 45 diabetic patients. Reductions in levels of inflammatory cytokine levels (e.g., TNF-$\alpha$ and IL-6) have also been observed following weight loss in obese patients [128]. Not only might caloric restriction result in histological improvement in NAFLD, but altered diet composition might prove beneficial as well. Ryan et al. [129] assigned 52 obese patients to a diet consisting of either 60% or 40% carbohydrates for 16 weeks. Patients in the lower-carbohydrate group had significantly greater reductions in ALT and steady-state glucose levels. In a retrospective study that examined whether the type of carbohydrate affected NAFLD, participants who consumed a larger percentage of fructose-containing products had a 2–3-fold increased risk of developing NAFLD [130]. Fructose-containing foods might predispose to the development of NAFLD by promoting lipogenesis,

hypertriglyceridemia, and insulin resistance [131]. However, studies examining diet and exercise have not revealed significant reductions in fibrosis.

Despite the lack of evidence documenting the association between diet and exercise and histologic improvement of NAFLD, these interventions should be first-line therapies for overweight and obese patients with NAFLD given the strong association between NAFLD and obesity. Various authors have recommended a target weight loss of greater than 7% [125, 132].

*Pharmacologic Therapy*

In trials examining obese patients, pancreatic lipase inhibition has been associated with weight reduction and improvements in insulin resistance and LDL profiles [133, 134]. A small case series reported a reduction in LFTs and improvement in histology in patients treated with a lipase-reducing agent [135]. Similarly, Hatzitolios et al. [136] demonstrated a significant reduction in LFTs and ultrasonographic appearance of steatosis in 21 patients treated with a lipase-inhibiting agent for 24 weeks. In another study, 13 subjects received sibutramine and 12 subjects received orlistat. After 6 months, both groups showed significant improvements in insulin resistance, LFTs, and ultrasonographic evidence of steatosis [137].

Several authors have also chosen to study mediators implicated in appetite regulation: cannabinoids and incretins. The cannabinoid system has been implicated in appetite regulation. When rats were fed with an inhibitor of the cannabinoid ($CB_1$) receptor, they demonstrated sustainable weight loss after therapy [138]. Recently, mice treated with oral rimonabant (cannabinoid receptor antagonist) showed reductions in hepatomegaly, hepatic steatosis, GGT, alkaline phosphatase, and TNF-$\alpha$ and showed higher levels of adiponectin [139]. In a large randomized trial, obese and overweight subjects provided with 20 mg rimonabant experienced a significant decrease in waist circumference, triglycerides, and LFTs, and improvements in HDL, adiponectin levels, and insulin resistance [140]. However, participants in the treatment group experienced clinically and statistically significant increases in depression and anxiety that prompted discontinuation of the study medication. Similar adverse events have been reported in recent trials that resulted in study discontinuation. For this reason, cannabinoid receptor antagonists have not been granted approval for use as treatment of the metabolic syndrome or NAFLD in Europe or the USA. This data suggests that cannabinoid receptor inhibition can reduce risk factors associated with NAFLD, but that the risks might outweigh the benefits.

Lower levels of glucagon-like peptide 1 (GLP-1), which promotes insulin release, and glucose-dependent insulinotropic polypeptide (GIP) are found in obese patients [141]. Ding et al. [142] showed that obese mice treated with exendin, a GLP-1 agonist, showed improvements in insulin resistance and decreases in histologic steatosis. Only one case report illustrates a similar effect in humans. A 54-year-old gentleman showed improvement in liver steatosis as measured by liver spectroscopy after 44 weeks of treatment with exenatide, a GLP-1-like peptide [143].

It is likely that improvement in liver histology seen with pancreatic lipase inhibitors and appetite-suppressive agents (i.e., sibutramine and rimonabant) are related to weight loss rather than direct effects of the agents on liver parenchyma. In patients who fail to lose weight with dietary modification and exercise, these classes of pharmacological agents represent a safe alternative before recommending bariatric surgery (Table 2). Larger human trials are needed to assess the efficacy and safety of GLP-1 agonists.

*Bariatric Surgery*

Bariatric surgery is generally recommended in patients with BMI greater than 40 kg/m$^2$ or in patients with BMI greater than 35 kg/m$^2$ who suffer from an obesity-related condition (e.g., hypertension, insulin resistance, hyperlipidemia, and obstructive sleep apnea). Surgical weight reduction is associated with significant improvements in biochemical and histologic markers of NAFLD [19, 144–156]. Dixon et al. [19] showed significant improvements in ALT, steatosis, inflammation, and fibrosis among a group of obese patients after laparoscopic adjusted gastric banding (LAGB). Kral et al. [144] demonstrated similar beneficial effects on these same markers in a series of 102 patients who underwent reoperation after biliopancreatic diversion. In a series of 185 patients who underwent bariatric surgery (both intestinal bypass and gastric banding procedures), Mathurin et al. [146] showed that patients had reductions in steatosis and BMI after 1 year of follow-up, but that these same patients had higher fibrosis scores at 1 year. Furthermore, patients with more severe insulin resistance and higher BMI showed persistence of steatosis after operation. While it is clear that bariatric surgery has beneficial effects on BMI and other metabolic parameters, there are reports that extreme weight loss can result in *increased* hepatic morbidity, including inflammatory changes and fibrosis [147, 148].

Bariatric surgery is an effective treatment for obesity in patients who do not experience adequate weight loss after lifestyle modifications and pharmacological therapies. In candidates appropriate for bariatric surgery, the patient's primary-care physician, hepatologist, and bariatric surgeon must collaborate during the perioperative period to ensure patient safety and effectively manage potential postoperative complications (e.g., dumping syndrome, vitamin deficiencies, and diarrhea).

**Table 2** Selected pharmacologic agents for the treatment of NAFLD-human data

| Class | Agent | Mechanism | Effect: LFTs | Effect: steatosis | Effect: inflammation/ fibrosis | Comment |
|---|---|---|---|---|---|---|
| Thiazolidinediones [142–148] | Pioglitazone | PPAR-$\gamma$ agonists → increased insulin sensitivity | ↓ | ↓ | ↓ | Small studies, but some randomized. Side-effects include weight gain, potential cardiovascular morbidity. |
| | Rosiglitazone | | ↓ | ↓ | ↓ | As above |
| Biguanides [153–156] | Metformin | Decreases hepatic gluconeogenesis; improves insulin sensitivity | ↓ | ↓ | ↓ | Small studies, one RCT compared metformin with vitamin E or diet therapy. Effect on histology unclear. Concern for hyperlactatemia |
| Bile acid sequestering agents [158, 159, 161, 176] | Ursodeoxycholic acid (UDCA) | Cholesterol-lowering; reduces inflammatory cytokine production | ↓ | ↓ | – | Small studies, one large RCT showed no benefit. Side-effects include gastrointestinal upset |
| Fibric acid [164, 167] | Clofibrate | Reduces cholesterol biosynthesis | – | – | – | |
| | Gemfibrozil | Inhibits lipolysis → decrease FFAs | ↓ | – | – | |
| 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors [163–166] | Atorvastatin | Reduces cholesterol biosynthesis | ↓ | ↓ | – | Small studies. Only retrospective study showed histologic improvement. No adverse effects observed |
| | Rosuvastatin | | ↓ | – | – | One small study, histology not assessed |
| Pancreatic lipase inhibitors [125–127] | Orlistat | Decreases absorption of FFA | ↓ | ↓ | – | |
| Norepinephrine-serotonin reuptake inhibitors [127] | Sibutramine | Promotes early satiety | ↓ | ↓ | – | |
| Cannabinoid receptor antagonists [130] | Rimonabant | Promotes early satiety | ↓ | ↓ | – | Human data revealed improvements in biochemical data, histology not assessed. Murine data revealed improvement in steatosis |
| Glucagon-like peptide-1 [133] | Exenatide | Stimulates insulin release | – | ↓ | – | Data from one human case report. Murine data showed improvement in steatosis |
| Vitamin E [172, 173, 175, 176] | | Antioxidant; free-radical scavenger | ↓ | ↓ | ↓ | Data inconclusive. May be associated with dose-related increase in mortality |
| Betaine [178–180] | | Antioxidant | ↓ | – | ↓ | Small trial |
| Angiotensin-receptor antagonists [184, 185] | Various | Blood pressure reduction; beneficial effect on fibrosis | ↓ | – | ↓ | Small studies. Murine models show improvement in inflammation and fibrosis |

## Insulin-Sensitizing Agents—The Thiazolidinediones

Insulin resistance plays a central role in the pathogenesis of NAFLD, and several authors have exploited this mechanism in designing therapeutic trials for NAFLD (Table 2). Agonists of the peroxisome proliferator-activated receptor-gamma (PPAR-$\gamma$) receptor, the thiazolidinediones, have been shown to improve insulin sensitivity and are also effective for the treatment of type II diabetes.

In an early study, troglitazone was evaluated in a series of ten women with biopsy-proven NASH. Most had reductions in ALT after 48 weeks of treatment, with four of seven patients experiencing modest reductions in necroinflammatory grade [149]. Troglitazone, while associated with improvement in LFTs and histology in patients with NASH, was associated with idiosyncratic, fulminant liver failure and was removed from the market in 2000 [150, 151].

Pioglitazone and rosiglitazone have also been studied for treatment of NAFLD. Promrat et al. [152], in a series of 18 patients, showed that treatment for 48 weeks with pioglitazone resulted in significant improvements in LFTs, steatosis, inflammation, and fibrosis. However, not all studies examining pioglitazone have shown improvement in fibrosis. A larger trial by Belfort et al. [153] randomized 55 subjects with impaired glucose tolerance and biopsy-proven NASH to a combination of dietary therapy and pioglitazone versus dietary therapy alone for 6 months. Subjects who received pioglitazone therapy showed significant reductions in LFTs, steatosis, and necroinflammation, but did not show improvement in fibrosis. Aithal et al. [154] published similar results in another randomized trial in which 61 nondiabetic patients with biopsy-proven NASH were randomized to 12 months of pioglitazone therapy or placebo and then underwent repeat liver biopsy. While the intervention group showed reductions in ALT, steatosis, hepatocellular injury, Mallory bodies, and fibrosis, only improvement in hepatocellular injury and Mallory bodies were *significantly* improved when compared with placebo. Of note, the improvement in fibrosis approached significance ($p = 0.05$). Similarly, treatment with rosiglitazone has resulted in improvement in both LFTs and histologic inflammation, but has not been shown to result in reduction of fibrosis [155, 156]. Ratziu et al. [157] randomized 63 patients with NASH to rosiglitazone or placebo for 12 weeks. Subjects receiving rosiglitazone had significant reductions in LFTs and steatosis, but no significant reduction in inflammation or fibrosis after repeat biopsy. More recently, Ratziu et al. [157, 158] reported 3-year follow-up data on 40 of these patients who underwent repeat biopsies. While there was improvement in steatosis, there was no improvement in NAS score or fibrosis.

Safety concerns remain regarding the use of thiazolidinediones for treatment of NAFLD. Beyond the risk of hepatotoxicity (largely negligible now that troglitazone has been removed from the marketplace), this class of medication is associated with weight gain (which can be in excess of 2 kg), which may be related to increased adipose tissue deposition rather than an increase in water weight [156]. Additionally, recent data suggests that rosiglitazone might be associated with an increased risk of myocardial infarction and cardiac death, although authors highlight methodological limitations to the study [159, 160]. Pioglitazone does not seem to carry this hazard, but may predispose at-risk patients to exacerbations of heart failure. This effect may be related to weight gain that is common in patients receiving this agent [161]. In short, thiazolidinediones can safely be recommended to the appropriate NAFLD patient (i.e., without history of congestive heart failure) for the treatment of hyperglycemia associated with type II diabetes. For patients with NAFLD, therapy with this group of medications can be used in patients who are resistant to nonpharmacologic therapies. However, careful analysis of the potential risks associated with thiazolidinediones must be considered prior to administering this medication. One recent review suggests that patients receiving thiazolidinedione therapy be treated for a minimum of 12 months [162]. This group of patients may show benefit via a reduction in LFTs and improvement in liver histology. The NAFLD community is eagerly awaiting the results of the PIVENS trial in which 247 nondiabetic patients with biopsy-confirmed NASH were randomized to receive pioglitazone, vitamin E or placebo for 96 weeks. The primary outcome will be improvement in NAS score. Results of this large trial have the potential to profoundly influence the ways in which gastroenterologists approach the use of thiazolidinediones for the treatment of NAFLD in nondiabetic patients [163].

### The Biguanide-Metformin

Several trials have also examined the role of metformin in the treatment of NAFLD (Table 2). Leptin-deficient mice given metformin show marked improvement in liver steatosis [164]. In an early study examining the role of metformin in the treatment of patients with NAFLD, Marchesini et al. [165] showed that treatment with metformin for 1 year resulted in improvement in LFTs. Nair et al. [166] followed 15 patients who received metformin for 1 year and demonstrated initial improvement in LFTs, but the effect was not sustained. Some patients showed improvement in steatosis (3/10) and fibrosis (1/10) after 1 year of treatment [166]. In a study including 36 patients with NASH randomized to combined metformin and dietary therapy for 6 months or dietary therapy alone, subjects receiving metformin had significant improvements in LFTs versus dietary therapy alone [167]. While a minority of patients underwent postintervention biopsy, those in the dual-therapy group who underwent biopsy showed no significant improvements in necroinflammatory activity and fibrosis compared with dietary therapy alone. Most recently, in a pediatric population, Nobili et al. [168] randomized 60 pediatric patients with steatosis only or NASH to metformin or dietary therapy. While LFTs, steatosis, and inflammation improved in both groups of subjects, there was no improvement in fibrosis.

While these studies revealed that metformin is associated with a reduction in LFTs and, in some patients, with reductions in inflammation and fibrosis, studies utilizing metformin have been limited by small sample sizes, small treatment effects, and short duration of follow-up. There still exists concern about the potential for lactic acidosis in patients treated with metformin. It is estimated that the incidence of lactic acidosis in patients taking metformin is

5/100,000 and is more common in patients predisposed to elevated lactic acid levels (e.g., congestive heart failure, renal insufficiency, and sepsis) [169]. Marchesini et al. [165] showed that, while the mean level of lactic acid rose during treatment with metformin, the mean value was not above the upper limit of normal. Nair et al. [166] reported similar results, with only 1 of 15 patients experiencing abnormal elevation in venous lactic acid. This agent should be utilized in a similar manner to the thiazolidinediones. In the appropriate patient with a hyperglycemia syndrome (i.e., without chronic renal insufficiency, congestive heart failure or sepsis), metformin can be used and may produce biochemical and histologic improvement of NAFLD.

### Ursodeoxycholic Acid (UDCA)

UDCA, an agent believed to have cytoprotective properties, resulted in improvements in biochemical and histological parameters in smaller trials [170–172]. Laurin et al. [170] compared therapy with ursodeoxycholic acid (UDCA) with gemfibrozil in a series of 40 patients with biopsy-proven NASH who underwent repeat biopsy after 1 year of therapy. Only the UDCA group showed significant reduction in LFTs and steatosis. In another trial, Santos et al. [171] randomized 30 patients to therapy with UDCA or placebo and found significant reductions in LFTs in the treatment group, but no changes in steatosis as detected by CT. On the other hand, Lindor et al. [173] published the largest trial to date, in which 166 patients with NASH were randomized to UDCA or placebo for 2 years. No significant reductions in steatosis, inflammation or fibrosis were observed in the treatment group.

Even though UDCA has a favorable side-effect profile, the available evidence from a large trial suggests that UDCA therapy does not result in histologic improvement (Table 2). Therefore, UDCA alone is not recommended for the treatment of NAFLD.

### 3-Hydroxy-3-Methylglutaryl-coenzyme A (HMG-CoA) Reductase Inhibitors (Statins) and Fibric Acid Derivatives

Statins are effective agents for the management of dyslipidemia and are first-line agents as outlined in the National Cholesterol Education Program Adult Treatment Panel III guidelines [174]. Gomez-Dominguez et al. [175] administered atorvastatin to obese patients with steatosis. After 12 months of therapy, patients generally showed reductions in LFTs, with normalization of LFTs in some patients. However, there was no significant change in steatosis. In another study, patients with biopsy-proven NASH were administered UDCA or atorvastatin. Both groups showed significant reductions in LFTs, but no reduction in inflammation or fibrosis as measured by CT after 6 months of therapy [176]. In contrast to these two studies, Ekstedt et al. [177] recently published a retrospective histologic review comparing 51 patients who did not receive statins with 17 patients who received a statin for an average of 6.1 years. The statin group showed a significant reduction in steatosis, but unlike the aforementioned studies, did not show significant reductions in LFTs. Four of 17 patients (24%) progressed to fibrosis in the statin group compared with 23/51 (45%) in the control group. Similarly, rosuvastatin was studied in a small prospective study of 22 patients with NAFLD. After 8 months of therapy, subjects showed a significant reduction in LFTs. However, liver histology was not assessed [178]. Similar results were noted in a study using fibric acid (gemfibrozil): patients who received gemfibrozil only showed an improvement of LFTs [179].

In general, the use of statins may be associated with asymptomatic elevations in liver LFTs, and in some cases, rare reports of severe hepatotoxicity have been noted [180]. However, these drugs are considered safe in patients with liver disease and also in patients with abnormal LFTs without intrinsic liver disease [181]. Other side-effects of statin therapy include myalgias and, rarely, myopathy. Current evidence suggests that statin therapy has pleiotropic effects on vascular endothelium, inflammation, and plaque stability that extend beyond cholesterol reduction and may benefit patients regardless of cholesterol level [182]. The current evidence suggests that therapy with statins might result in reductions in LFTs, but evidence demonstrating histologic improvement is lacking (Table 2). Until larger studies are performed in patients with NAFLD, statins should not be administered for primary treatment of NAFLD, but rather as a cornerstone of treatment for risk factor modification.

### Antioxidant Therapy

Vitamin E and betaine have been studied for their antioxidant potential in the setting of NAFLD [183]. Kugelemas et al. [184] randomized 16 patients with NASH to combined dietary and vitamin E therapy or dietary therapy alone for 6 weeks. Subjects in the combined therapy group showed reductions in IL-6, suggesting that vitamin E therapy might reduce inflammatory mediators implicated in the pathogenesis of NAFLD. Hasegawa et al. [185] performed a study in which 12 patients with NASH and 10 patients with steatosis were randomized to dietary therapy or vitamin E therapy. Patients with NASH experienced nonsignificant reductions in LFTs after 6 months of dietary therapy, but experienced significant reductions in LFTs after therapy with vitamin E. On the other hand, subjects with steatosis showed significant reductions in LFTs after dietary therapy, but did not benefit from therapy with vitamin E. None of the 12 patients with NASH who

underwent posttreatment biopsy showed worsening of histology, while 6 of 9 patients showed improvements in steatosis and 5 of 9 showed improvements in fibrosis and inflammation.

Combination therapy with vitamin E has also been studied. Sanyal et al. [186] compared the combination of vitamin E and pioglitazone with vitamin E alone in a randomized trial in 20 NASH subjects. Subjects who received combination therapy showed improvements in steatosis, inflammation, and fibrosis, while subjects in the vitamin E group showed improvement in steatosis alone. In another randomized trial, Harrison et al. [187] randomized 45 patients with biopsy-proven NASH to combined vitamin E and vitamin C therapy or placebo. Combined therapy did not result in biochemical improvement or reduction in inflammation on repeat biopsy, but did results in improvement in fibrosis. In a study of 50 patients with NASH given vitamins E and C for 5 years, Shimada et al. [188] showed improvements in LFTs. Five of ten patients who underwent repeat biopsy showed improvement in inflammation and three of ten showed improvement in fibrosis. Madan et al. [189] compared patients with biopsy-proven NAFLD on placebo, UDCA or a combination of UDCA and vitamin E. Only ALT levels were significantly reduced in the combination therapy group compared with the other groups. Histology was not assessed in this trial.

Song et al. [190] reported that mice showing diet-induced steatosis had reductions in steatosis after treatment with betaine, a choline-derived metabolite, and also demonstrated increased levels of adenosine-monophosphate-activated protein kinase. In addition, in a small trial of ten subjects with NASH, oral betaine was associated with reductions in ALT and improvements in inflammation and fibrosis [191, 192]. Abdelmalek et al. [193] reported improvement in steatosis, but there was no additional histologic or biochemical (adiponectin or proinflammatory cytokines) improvements in a group of 55 patients with biopsy-proven NASH who underwent 12 weeks of therapy with betaine.

The role of vitamin E and betaine in the treatment of NAFLD is still unclear. The trials cited above are limited by small sample sizes, short duration of treatment, and endpoints that do not uniformly include histologic assessments (Table 2). While vitamin E is generally well tolerated, it has been associated with rare and potentially worrisome side-effects, including coagulopathy (usually in patients with underlying coagulation defects) [194]. Furthermore, a meta-analysis raised the concern about a dose-dependent increase in mortality associated with vitamin E [195]. The author commented that the studies chosen for the meta-analysis had methodological differences, contained small numbers of subjects, and suffered from incomplete data collection. In summary, it seems most prudent to use this vitamin E at the lowest dose possible for improvement in LFTs and histology. Larger, randomized trials of vitamin E and betaine are necessary to define their place in the armamentarium of NAFLD therapy.

### Angiotensin-Receptor Antagonists

The renin-angiotensin-aldosterone system (RAAS) is a well-known target of antihypertensive therapy. Recently, the RAAS has been implicated in the pathogenesis of hepatic fibrosis. Yoshiji et al. [196] examined the effect of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin-receptor blockers (ARB) in rats injected with pig serum to stimulate NAFLD. Rats given candesartan (an ARB) or perindopril (an ACEI) for 8 weeks showed marked reductions in markers of fibrosis (e.g., hyaluronic acid and TGF-$\beta$) as well as histologic fibrosis. Importantly, the potential benefit of ACEI and ARB therapy in NAFLD was demonstrated in a randomized trial in which patients were randomized to placebo, the ARB telmisartan ($n = 28$) or the ARB losartan ($n = 26$) for 20 months. Both treatment groups showed significant reduction in ALT, NAS score, and insulin resistance compared with placebo [197]. In a case series of seven hypertensive patients with biopsy-proven NASH, Yokohama et al. [198] showed that treatment with losartan for 48 weeks resulted in improvement in LFTs, reduction in markers of fibrosis (hyaluronic acid, type IV collagen, and procollagen) and inflammation (e.g., TGF-$\beta$), and improvement in histologic inflammation and fibrosis.

Preliminary data from small studies is promising in that treatment with ACEIs and ARBs has resulted in histologic improvement in NAFLD (Table 2). However, larger prospective studies of ACEIs and ARBs are required to further define the relationship between the RAAS and NAFLD and their role in the treatment of NAFLD. Until such time, ACEIs and ARBs should continue to be used as therapy for hypertension, especially in patents with diabetes, microalbuminuria, and cardiac dysfunction.

### Transplantation

Some patients with NASH may develop cirrhosis, and a subset may progress to decompensated cirrhosis and thus require liver transplantation [199]. Interestingly, NASH has been shown to recur in up to one-third of patients following transplantation [199, 200]. In one case, liver biopsy performed 66 weeks after transplant revealed presence of steatohepatitis in a previously normal liver [188].

### Future Therapy for NAFLD

Biotechnology has revolutionized the pharmacologic landscape and offers promise for the development of new

agents to treat liver disease. Several studies are currently enrolling patients with NAFLD [201]. A phase II, randomized, placebo-controlled trial is currently underway evaluating an oral caspase inhibitor, GS 9450 (Gilead, Foster City, CA, USA), in subjects with NASH. Caspases are proteases essential for apoptosis, a process that is implicated in the development of NASH. GS 9450, therefore, is hypothesized to suppress dysregulated apoptosis. Another study is enrolling patients with NASH who will be randomized to placebo or TRO19622 (Trophos, Marseille, France), an agent believed to promote cellular survival in neural tissue, cardiac muscle, and hepatic parenchyma [202]. This agent has been shown to bind to mitochondrial membrane proteins that play roles in apoptosis, resulting in suppression of apoptosis [203]. A novel phosphodiesterase inhibitor (ASP9831; Astellas Pharma, Inc., Japan) is being evaluated in a phase II clinical trial study. CP-945598, a cannabinoid-1 receptor antagonist, has been undergoing phase III trial in subjects with NASH who are receiving placebo or CP-945598 (Pfizer, New York, USA) [204]. Lastly, recombinant leptin is also being evaluated for the treatment of NASH in a nonrandomized trial [205]. Hopefully, these studies will provide more data regarding the clinical effectiveness of pharmacologic therapy for NAFLD.

## Conclusions

NAFLD is becoming an increasingly common cause of elevated LFTs. This parallels the increasing prevalence of the metabolic syndrome and obesity. Research studies have provided insight into the epidemiology, pathogenesis, and treatment of NAFLD, but there is ample room for further research and discovery. Recent advances have revealed the potential for noninvasive measures (e.g., TNF-α, adiponectin, and HA) of inflammation and fibrosis that might circumvent the need for liver biopsy. Currently, risk factors modification (i.e., weight loss, dyslipidemia, and insulin resistance) remains the cornerstone of therapy for NAFLD. Several trials have demonstrated the efficacy of insulin-sensitizing, antihyperlipidemic, and antioxidant agents, among other therapies, but larger trials are required if these are to become standard of care.

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